Evaluation of human induced pluripotent stem cell (hiPSC)-derived tri-culture as *in vitro* model for neurodegeneration and neuroinflammation

Katerina Pitsa, Leo Smit, Abeera Popalzij and Shushant Jain

Ncardia BV Leiden, The Netherlands, support@ncardia.com, www.ncardia.com

Abstract no. #277

Background

Despite significant advances in neuroscience research, the lack of physiologically relevant, human-based in vitro models remains a major limitation in studying neuroinflammation and neurodegenerative disease mechanisms, particularly tauopathies. Conventional preclinical models often fail to capture the complex cellular interactions between neurons, astrocytes, and microglia that drive inflammatory and degenerative processes in the human brain, creating an unmet need for more translatable platforms.

To address this gap, Ncardia developed a physiologically relevant human iPSC-derived tri-culture model aimed at enabling robust investigation of neuroinflammation and neurodegeneration in a controlled in vitro setting.

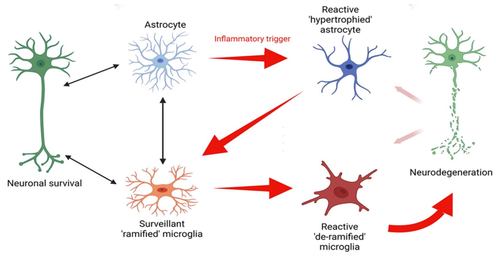

Figure 1. Cross-talk between neurodegeneration and neuroinflammation

Neuronal homeostasis relies on astrocytes and microglia activation status, that is disturbed in pathogenic conditions as neurodegeneration.
Diseased neurons secrete factors that activate microglia and astrocytes, initiating a cascade of inflammatory triggers that induce further neurodegeneration and neuroinflammation.
Adapted from: Ullah, F., Gamage, R., Sen, M.K. et al. The Effects of Modified Curcumin Preparations on Glial Morphology in Aging and Neuroinflammation. Neurochem Res 47, 813–824 (2022). https://doi.org/10.1007/s11064-021-03499-4

Methods

Neurons, astrocytes, and microglia derived from human iPSCs were combined to establish tri-cultures and treated with recombinant mutant Tau pre-formed fibrils (PFFs) to induce a tauopathy-like phenotype. Quantification of Tau aggregation and microglial phagocytosis was performed using high-content imaging, while cytokine release and soluble Tau levels were measured using MSD and ELISA assays, respectively.

The tri-culture model was characterized for cell-type composition and microglial activation, capturing key biological processes observed in the human brain, including pro-inflammatory cytokine release and phagocytic activity.

Ncyte® Microglia – Cytokine release and phagocytic activity

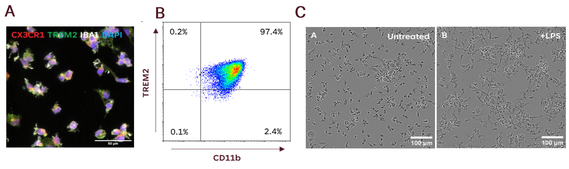

Figure A) Immunofluorescent staining of Ncyte® Microglia with markers IBA, TREM2 and CX3CR1, co-stained with DNA marker DAPI, on day 7 post-thaw.
Figure B) Flow cytometry analysis of Ncyte® Microglia showing TREM2/CD11b double positive cells (97.4%).
Figure C) Brightfield images of Ncyte® Microglia. A) Untreated microglia and B) Microglia treated for 18hrs with LPS

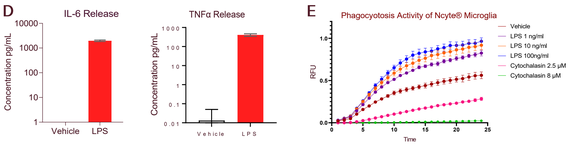

Figure D) Release of cytokines IL-6, TNFα on day 7 post-thaw in Ncyte® Microglia treated for 18hrs with LPS as measured by Mesoscale Discovery.
Figure E) Day 7 Ncyte Microglia were incubated with pHrodo Green labelled E. coli particles for 24 hours +/- cytochalasin D or +/- LPS control. Images were acquired every 30 mins on the Incucyte® looking at green fluorescence and phase contrast. The graph displays the fluorescence intensity per cell displaying degree of phagocytosis per cell.

Ncyte® Neural Mix – Mature neurons and astrocytes co-culture expressing SYN and PSD-95

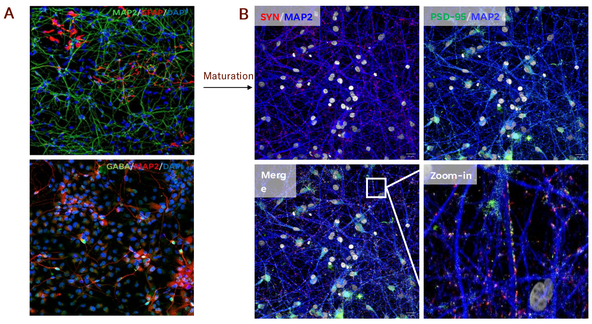

Figure A) Fluorescent images of Ncyte Neural Mix with >70% of βIII-Tubulin+ neurons, ≥ 50% of s100β+ astrocytes in non-neuronal population. Presence of glutamatergic and GABAergic neurons

Figure B) Ncyte neural mix can be matured and maintained in culture and exhibit synaptic maturation as evident by the colocalization of pre-synaptic marker synaptophysin-SYN (in red), post-synaptic marker PSD-95 (in green) and neuronal marker MAP2 (in blue). Nuclei were stained with DAPI (in grey).

Miniaturized tauopathy assay based on Ncardia's human triculture system

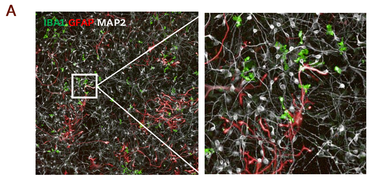

Figure A) Fluorescent images from Ncyte tri-cultures stained for microglia (IBA1 in green), astrocytes (GFAP in red) and neurons (MAP2 in white). Nuclei were stained with DAPI (in grey).

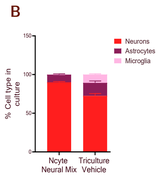

Figure B) Tri-culture is composed of neurons (~70%), astrocytes (~17%) and microglia (~11%)

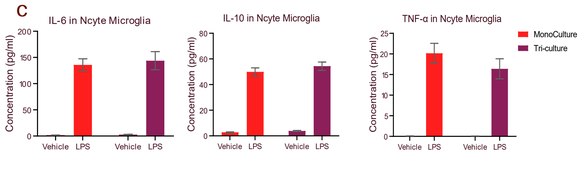

Figure C) Under basal condition, Ncyte microglia do not secrete key pro-inflammatory cytokines either in mono or tri-culture systems. Upon stimulation with LPS, Ncyte® Microglia exhibit a strong pro-inflammatory reaction both in monoculture as well as in a tri-culture system comprising of neurons, astrocytes, and microglia. LPS treatment significantly increases the production of the key inflammatory mediators IL-6, IL-10 and TNF-α in both models, as measured by Mesoscale Discovery.

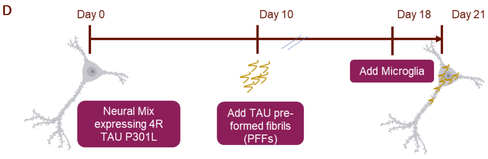

Figure D) Schematic of the AD model using iPSCs derived neurons, astrocytes and microglia treated with TAU pre-formed fibrils (PFFs). Day 10 post thaw co-cultures expressing 4R TAU P301L were treated with TAU PFFs. On day 18, Ncyte microglia were added. Co-culture of neurons, astrocytes and microglia was kept for 21 days for endpoint analysis of phospho-TAU (pTAU), MC-1 positive TAU in neurons (by ICC-IF).

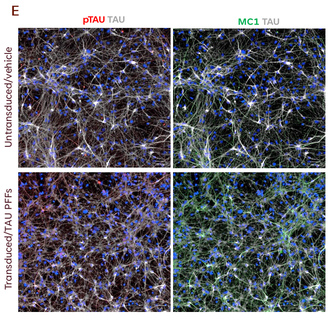

Figure E) Fluorescent images of untransduced and transduced day 21 post-thaw tri-cultures stained for phospho-TAU (red), MC-1 (green), TAU (grey) and DAPI (blue). Transduced/TAU PFFs treated tricultures show increased levels of TAU, pTAU and MC-1 TAU.

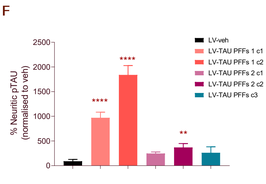

Figure F) High content-based quantification of the normalized parameters (pTAU puncta counts) of transduced tricultures (in black) compared to transduced tricultures treated with TAU PFFs.

Conclusion

Ncardia has developed a human iPSC-derived tri-culture model that enables modulation and quantitative assessment of neuroinflammation and neurodegeneration in vitro. This platform provides critical insight into microglia–neuron and microglia–astrocyte interactions that govern recognition of apoptotic neurons, inflammatory signalling, and neuronal dysfunction—key processes underlying disease progression and highly relevant for translational neuroscience research and drug discovery.